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Rapid and Efficient Synthesis of Peptide Fragments containing α-Aminoisobutyric acid using Fmoc-Amino acid Chlorides/Potassium salt of 1-Hydroxybenzotriazole

Vommina V. Suresh Babu* and Hosahudya N.Gopi

Department of Studies in Chemistry, Central College Campus,
Bangalore University, Bangalore - 560 001, India.

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Abstract : The synthesis of peptides containing multiple Aib residues was accomplished using Fmoc-Aib-Cl in presence of KOBt. As no additional base was added, the duration of coupling reactions could be extended. Thus, the synthesis of the alamethicin 1-4 fragment, Aib-Pro-Aib-Ala, the emerimicin 2-6 fragment, Aib-Aib-Aib-Val-Gly and the Aib tetramer, Fmoc-$(Aib)_4$-OBzl were accomplished in good yield and purity.

Linear, amphipathic peptaibol antibiotics such as alamethicin and the emerimicins contain many sterically hindered α ,α-dialkylamino acids such as Aib, Eta[1]. They are known to form voltage dependent ion-conducting pores in lipid bilayer membranes[2]. Their chemical synthesis and homosequences such as Aib-Aib-Aib are known to be difficult under normal mild conditions.[2a-d] The coupling of Boc-/Z-Aib/Boc-Leu-Aib with Aib-OMe/Aib-Aib-Pro-OMe employing DCC or DCC/HOBt or BOP/other related agents is reported to be incomplete even after 24 hr and results in 45-55% yields of peptides.

Far from being "over-activated" and therefore prone to side reactions, the acid chlorides of Fmoc-amino acids are found to be rapid, efficient, optically pure, shelf stable under anhydrous conditions and racemization free coupling agents.[3a-c] The acylation reactions employing them can be carried out using KOBt in place of 1:1 mixture of HOBt and a base[4]. The use of an equimolar quantity of organic base is known to result in 2-(9-fluorenylmethoxy)-5(4H)-oxazolone formation and enantiomerization if the activated species is allowed to stand for sufficient time.[5] The use of KOBt as an additive eliminates the formation of oxazolone. Consequently the duration of coupling reactions can be extended. The danger also of premature deblocking of the Fmoc group by base is also circumvented.[6]

In this report we find that acid chlorides of Fmoc-Aib results in efficient incorporation of the acid moiety. The coupling of Fmoc-Aib-Cl[7] in the presence of equimolar quantities of KOBt in CH_2Cl_2 is complete within 20-30 min. (as monitered by TLC). Thus, Fmoc-Aib-X-OMe(X = Val/Ala) was obtained in good yield and purity.[8] KOBt was used to convert hydrochloride salts of amino acid esters to their free amines *in situ*. Extending the duration of coupling reaction to 45-60 min., allowed the efficient synthesis of the homosequences containing two Aib residues (Table1). Parallel studies showed that the use of Fmoc-Aib-OPfp[9]/HOBt, Fmoc-Aib-OTcp[9]/HOBt/base and Fmoc-Aib-NCA,[9] Fmoc-$(Aib)_2O$[9] as coupling agents for the synthesis of Fmoc-Aib-Aib-OBzl resulted in 42%, 28%, 35% and 40% yields respectively even after prolonged reaction times. However, the coupling of Fmoc-Aib-F/diisopropylethyl amine to Aib-OBzl resulted in 69% of the corresponding peptide. Fmoc-amino acid chlorides/KOBt, were employed further for the synthesis of the protected alamethicin 1-4 fragment, Fmoc-Aib-Pro-Aib-Ala-OBzl[10] and emerimicin (III & IV) 2-6 pentapeptide fragment, Fmoc-Aib-Aib-Aib-Val-Gly-OBzl.[11] A coupling time of 30-65 min. and 30 min. for deprotection of Fmoc-group using 4-aminomethylpiperidine was maintained throughout the synthesis. All intermediates as well as the final product are obtained as pure crystalline compounds and not one chromatographic purification was needed. The purity of the peptides, as indicated by HPLC, was satisfactory. In the present study, the efficacy of Fmoc-Aib-Cl/KOBt was finally demonstrated by the synthesis of tetramer of Fmoc-Aib-Aib-Aib-Aib-OBzl in satisfactory yield (69%) and

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purity[12]. Thus, Fmoc-Aib-Cl can be prepared easily, shelf stable under anhydrous conditions for several

Table 1 **Fmoc-Aib dipeptides**

Peptide	Yield (%)	M.P ^{0}C	R_fB	R_fC
Fmoc-Aib-Aib-OMe	85	70-71	0.67	0.77
Fmoc-Aib-Aib-OEt	81	155-157	0.64	0.76
Fmoc-Aib-Aib-OBzl	83	131-132	0.62	0.79

days, less expensive compared to Fmoc-Aib-OPfp, Fmoc-Aib-NCA and has been demonstrated to be an efficient coupling agent in the presence of KOBt for the synthesis of homosequences such as Aib-Aib.

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1. Abbreviations and Notes: **Aib**, α-aminoisobutyric acid; **EtA**, α-ethyl alanine; **PyBOP**, (1H-1,2,3-benzotriazol-1-yloxy)tris(pyrrolidino)-phosphonium hexaflurophosphate; **KOBt**, potassium salt of 1-hydroxybenzotriazole; TLC analysis using (i) Ethyl acetate:hexane :: 35:65; (ii) $CHCl_3$:methanol:acetic acid :: 40:2:1; (iii) $CHCl_3$: methanol :: 9:1; (iv) n-butanol : acetic acid : water :: 4:1:1 and (v) n-butanol : acetic acid : water :: 4:1:5 and designated as R_fA, R_fB, R_fC, R_fD and R_fE. HPLC analysis on Waters LC3000 with 484 tunable UV detector, 745 datamodule and Deltapack C-18 Column (3.9x300 mm) using isocratic 65% methanol in water, U.V. at 252 nm, flow rate, 0.75 ml/min.

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7. **Fmoc-Aib-Cl**: A mixture of Fmoc-Aib (1 mmole) and freshly distilled thionyl chloride (1ml) in CH_2Cl_2 was stirred for 24 hr under anhydrous conditions, evaporated to dryness and isolated as a solid by adding CH_2Cl_2/hexane. Yield, 84%. M.P., 164-165°; R_fA, 0.65. It is converted to its methyl ester and used for analysis. Anal. Calcd. for $C_{20}H_{21}O_4N$ (339.39): C, 70.78; H, 6.23; N, 4.13%. Found : C, 70.49; H, 6.28; N, 4.08%.

8. **Fmoc-Aib-Ala-OBzl**: Yield, 81%; M.P.121-122°; $[\alpha]^{25}_D$-19.3° (c 1, CH_2Cl_2), R_fB, 0.6; R_fC, 0.75; **Fmoc-Val-Aib- OBzl**: Yield, 80%; M.P., 184-185°; $[\alpha]^{25}_D$-20.6° (c 1, CH_2Cl_2), R_fB, 0.59; R_fC, 0.74; **Aib-Ala-OBzl** : M. P., 178^0 ; **Val-Aib-OBzl** : M.P., 168-170^0.

9.**Fmoc-Aib-pentafluorophenyl ester**: Yield, 91%; M.P., 133-135°;R_fB, 0.71; **Fmoc-Aib-2,3,5-trichlorophenyl ester**: Yield, 86%; M.P., 147-148°; R_fB, 0.69; **Fmoc-(Aib)$_2$O**: Yield, 73%, M.P., 93-94°; R_fB, 0.69; **Fmoc-Aib-F**: M.P., 120-121°, R_fA, 0.72; Elemental analysis of ester, anhydride, and acid fluoride is satisfactory. Fmoc-Aib-N-carboxyanhydride obtained as a gift sample from Dr.A.Loffet, Pro-peptide, France.

10. **Fmoc-Aib-Pro-Aib-Ala-OBzl:** M.P., 68-69°; $[\alpha]^{25}_D$ + 30.1° (c1, CH_2Cl_2); R_fB, 0.72; R_fC, 0.6; R_t 6.73 min.;Amino acid analysis : Aib, 1.89 (2); Pro, 1.01 (1); Ala, 0.97 (1). Anal. Calcd. for $C_{38}H_{44}N_4O_7$ (668.79) : C, 68.24; H, 6.63; N, 8.38%. Found : C, 67.79; H, 6.74; N, 8.41%. **Pro-Aib-Ala-OBzl**; M.P., 165-166°; R_fD, 0.55; R_fE, 0.46; **Fmoc-Pro-Aib-Ala-OBzl**: M.P.,75-77°;$[\alpha]^{25}_D$ - 25.2° (c 1, CH_2Cl_2), R_fB, 0.43; R_fC, 0.58.

11. **Fmoc-Aib-Aib-Aib-Val-Gly-OBzl**: M.P., 88-90°; $[\alpha]^{25}_D$ + 10° (c 1, CH_2Cl_2); R_fB, 0.60; R_fC, 0.65; R_t 7.9 min; Amino acid analysis : Aib, 3.09(3); Val, 1.01(1); Gly, 0.98(1). Anal. Calcd. for $C_{41}H_{52}N_5O_8$ (742.89) : C, 66.29; H, 7.05; N, 9.43; Found : C, 65.89; H, 7.41; N, 9.18%. **Fmoc-Aib-Val-Gly-OBzl**: M.P., 130-131°; $[\alpha]^{25}_D$ + 32.4° (c1, CH_2Cl_2); **Fmoc-Aib-Aib-Val-Gly-OBzl**: M.P., 144-145°; $[\alpha]^{25}_D$ + 15.4° (c1, CH_2Cl_2); R_fB, 0.45; R_fC, 0.50 ; **Aib-Val-Gly-OBzl** : M.P., 160-161^0 ; **Aib-Aib-Val-Gly-OBzl** : M.P., 170-172^0.

12. **Fmoc-(Aib)$_4$-OBzl**: M.P., 70-72^0; R_fB, 0.50; R_fC, 0.63; **(Aib)$_2$-OBzl**: M.P., 184-186°; R_fD, 0.40, R_fE, 0.57; **Fmoc-(Aib)$_3$-OBzl**: M.P., 78-80°; R_fB, 0.54, R_fC 0.60 ; **(Aib)$_3$-OBzl** : M.P., 175-176^0; R_fD, 0.58; R_fE, 0.49.